The Fifth NIAID Workshop in Medical Mycology: Epidemiology

Duke University
Durham, North Carolina
August 13-16, 2000

PREFACE

The NIAID has recognized medical mycology as an area in need of development. An Institute-sponsored workshop on "Mycology Research in the 1990's" in Chicago, Illinois, 28-29 September 1991 addressed the increasing importance of medical mycology. Twenty medical mycologists from throughout the United States were invited to discuss the issues and to conceptualize and condense the active research areas into topic areas in need of development.

Five areas were targeted for focus. These were molecular mycology, diagnosis and treatment, immunology, antigen structure and function, and epidemiology. Each of these five topic areas was targeted for development into a separate workshop/minisymposium, co-sponsored by the NIAID and educational grants raised by the medical mycological community.

"Molecular Medical Mycology," the first workshop in the series, was held in Minneapolis, Minnesota on 24-26 June, 1993 and chaired by Dr. Paul T. Magee. One hundred and forty-seven mycologists attended and exchanged ideas. A key to the success of information exchange was the utilization of "break out" sessions that provided an informal setting for free exchange of ideas, an opportunity for a more active involvement for all of the participants, and an environment fostering new collaborations.

"Molecular and Immunologic Approaches to the Diagnosis and Treatment of Systemic Mycoses," the second workshop in the series, was held on the campus of Northern Arizona University, Flagstaff, Arizona, 8-11 June 1994. The workshop format was modeled after the first in the series, and was attended by 80 registrants. Drs. John Galgiani and Michael Pfaller chaired this event whose findings are still timely.

"Immunology in Medical Mycology (Part 1 of 2): Antigenic Peptides, Glycobiology and Vaccines," the third workshop in the series, was held at the Yellowstone Conference Center, Big Sky, Montana, 7-9 September 1995. The workshop format was modeled after the first two in the series and was attended by 90 registrants. Drs. Rebecca Cox, Jim Cutler, and George Deepe chaired the workshop whose findings were summarized in ASM News 62;81-84, 1996.

"Immunology in Medical Mycology (Part 2 of 2): Host Responses to Fungi," the fourth workshop in the series, was held at Granlibakken Conference Center, Lake Tahoe, California, 20-23 August 1997. The workshop format was modeled after the previous three in the series and was attended by 75 registrants. Drs. Thomas Kozel and Juneann Murphy co-chaired the workshop along with session chairs Drs. Arturo Casadevall and Jack Sobel.

"The Fifth NIAID Workshop in Medical Mycology: Epidemiology," which completes the five workshop series that was proposed in Chicago, was held at the R. David Thomas Center, Duke University, Durham, NC, 13-16 August 2000. The workshop format was modeled after the previous four in the series and was attended by 81 registrants. Dr. John Perfect chaired the workshop along with session chairs Drs. George Kobayashi, Michael Pfaller, John Taylor, and David Warnock.

I believe the workshop series was successful in accomplishing the stated goals, and that this success is representative of the field of medical mycology whose time has come. Finally, I would like to thank all those who contributed to the success of these workshops, including all of the participants, organizing and writing committees.

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INTRODUCTION

The workshop was designed to address the epidemiology of fungal diseases from a worldwide perspective and the implications for research. The goals of the workshop were to 1) explore the incidence and prevalence of fungal diseases around the world and consider those implications for future research; 2) identify any gaps in the general area of fungal epidemiology that could represent research opportunities, and 3) interface with investigators from other fields and other countries for interactive learning. Six theme and ten research presentations in five sessions reported representative research approaches in the field, and several presentations highlighted parallel developments in related fields. Five "break out" sessions of approximately 11 participants each were led in discussion by facilitators who summarized the results in separate "at large" sessions.

Key Concepts

- Epidemiologic studies of the mycoses would be greatly enhanced by: refined and validated case definitions; standardized and validated DNA fingerprinting methods; a worldwide web-accessible fingerprinting database; and, the creation of new networks to accrue risk factor, incidence, and public health cost data.

- Surveillance and risk assessment should be continued in both the community and hospital and on a national basis as well as within individual medical centers.

- Mycologists and public health officials in other countries should work together to determine the global incidence and attributable costs of fungal infections given that Aspergillus and Candida are likely to be globally emerging pathogens linked to medical advances.

- More rapid, effective diagnostic tests are urgently needed, especially for aspergillosis and candidiasis, not only for recognition and treatment of the diseases, but also for ascertaining real incidence and attributable costs.

- Host and pathogen genomics hold great potential to capitalize on risk factor analyses, diagnostic test development, cross species comparisons, therapeutic and vaccine investigations, and analysis of endemic or opportunistic mycoses.

- Better prevention methods are needed for fungal diseases, including vaccines, potent new antifungal drugs (fungicidal) for prophylaxis and treatment, and environmental control strategies.

SURVEILLANCE

Current Status

Over the past 20 years, fungal diseases have emerged as an important public health problem, largely as a result of a dramatic increase in the size of the population at risk. AIDS accounts for much of this increase, but other factors, such as the widespread use of immunosuppressive agents in cancer treatment and transplantation, have also contributed. Analysis of U.S. National Center for Health Statistics (NCHS) death records showed that fungal infections were the seventh most common cause of infectious disease-related mortality in 1992, and that mycotic disease-related fatalities had increased more than 3-fold since 1980. NCHS data also showed that, in 1994, fungal diseases resulted in 30,000 hospitalizations, and accounted for the fourth highest annual percentage increase (10%) since 1980.

Surveillance is the systematic collection, analysis, and interpretation of outcome-specific data for use in public health practice. Various surveillance systems have been used to investigate fungal diseases. Sentinel systems, such as the SENTRY antimicrobial surveillance program and the ARTEMIS global antifungal surveillance program, have proved useful to monitor the emergence of non-Candida species as causes of nosocomial bloodstream infection, and to follow trends in azole antifungal drug resistance among bloodstream isolates. However, sentinel surveillance systems may not be a truly representative sample of all hospitals.

Passive surveillance systems are not ideal for fungal infections. Because these diseases are not notifiable, there is minimal incentive to report cases. Even when volunteer networks are formed, such as those organized by national and international medical mycology societies, passive surveillance underestimates incidence rates, and may lead to inaccurate description of the epidemiology of these diseases.

Active surveillance for fungal disease is expensive and often difficult to conduct, but it has enabled accurate population-based incidence rates for several invasive fungal infections, such as candidemia and cryptococcosis. This form of surveillance has also enabled better risk factor studies to be conducted because the cases detected are more truly representative of the population.

Whatever surveillance system is chosen, the quality of the data generated is dependent on a defined population, a clear case definition, a mechanism for reporting, and a sufficient incentive for all participants to conduct the surveillance. For fungal diseases, several of these elements present distinct challenges. There are few standardized case definitions for invasive fungal infections and the limitations of current diagnostic tests for some diseases, such as aspergillosis, remain a major problem in developing such definitions. Fungal infections, especially the community-acquired endemic mycoses, result in a wide spectrum of clinical manifestations, ranging from asymptomatic to mild to severe, life-threatening illnesses. Therefore, determining the overall burden of infection is very difficult and surveillance usually concentrates on determining the burden of severe disease.

Continued surveillance for fungal diseases is essential to improve our understanding of their epidemiology. Surveillance will provide critical information that will enable the pharmaceutical industry and academic research institutions to develop clearer research priorities for the study of these diseases. In order to conduct better surveillance, it will be essential to devise improved diagnostic tests, to follow rigorous epidemiologic methods, and to have adequate support from public health agencies.

Recommendations

- Establish networks to conduct active population-based and sentinel surveillance as well as risk factor and cost analysis studies.

- Encourage mycologists and public health officials in other countries to work together to determine the global incidence and attributable costs of fungal infections given that Aspergillus and Candida are likely to be globally emerging pathogens linked to medical advances.

- Improve the understanding of the transmission of aspergillosis inside and outside the hospital environment, in order to develop improved prevention and control strategies.

RISK FACTOR STUDIES

Current Status

Risk factor studies for invasive mycoses are essential in clinical practice to help make predictions in the use of diagnostics, creation of prophylactic and empiric antifungal strategies, and understanding intensity of treatment regimens. There are at least three major immunosuppressed patient populations with defined risk factors: (1) bone marrow transplantation (BMT) and chemotherapeutic neutropenia; (2) HIV infection; and (3) solid organ transplants.

BMTs and those patients with hematologic malignancies receiving high-dose chemotherapy are at particularly high risk for fungal infections with *Candida* and *Aspergillus* species although less common fungi can produce infection, e.g., *Fusarium* species, *Pseudallescheria boydii*, *Cryptococcus neoformans*, *Trichosporon beigelii*, dematiaceous fungi and Zygomycetes. It is important to emphasize that not all neutropenic patients are at the same risk for fungal infection. Risk of fungal infection depends on depth and duration of neutropenia, repeated chemotherapeutic cycles, intensity of mucosal disruption, effects of corticosteroids and modulation of cytokines. Two shifting trends in fungal infections within BMTs are: (1) increasing incidence of candidiasis with non-*albicans Candida* species; and (2) bimodal distribution of aspergillosis in either neutropenic or post-engraftment phase. It is apparent that the risk of deep-seated mycoses is at least 10 times greater for allogeneic versus autologous BMT. Within the BMT group there are subsets of risk factors depending on age, HLA mismatch donor, acute GVHD, underlying disease, corticosteroid use, neutropenia, and environmental changes. Treatment of established fungal infections remains difficult but treatment goals are: (1) stabilize the infection during neutropenia and continue treatment after engraftment; (2) establish diagnosis of infection early; and (3) use prophylactic and empiric antifungal strategies in certain high risk patients. Several decades of clinical experience with progressive HIV infection have confidently defined the risk factors associated with opportunistic fungal infections in AIDS. These factors include: (1) declining CD4 counts; (2) HIV viral load; and, (3) exposure to the fungus. From mucosal candidiasis to invasive mycoses (such as cryptococcosis, histoplasmosis, coccidioidomycosis, pneumocystosis, and penicillosis), the rates of fungal infections can range from 60-90% to 6-30% respectively, and can complicate management of AIDS. In the recent era of highly active antiretroviral therapy (HAART), there has been a dramatic and consistent drop in opportunistic infection including all mycoses and drug-resistant fungal strains. Although risk of fungal infections in certain populations under HAART is reduced, it is clear that several populations will remain at high risk: (1) untreated patients presenting with AIDS; (2) those initiating antiretroviral therapy; and, (3) patients with antiretroviral drug-resistant disease.

The technical ability to transplant solid organs and prevent their rejection has been a major medical achievement in the last several decades. However, there remain certain complications to success, and fungal infections are one of these complicating factors. Incidence of fungal infections in organ recipients has been reported to be: renal (1.4-14%); heart (5-21%); liver (7-42%); lung and heart/lung (15-35%); small bowel (40-59%); and, pancreas (18-38%). Within each transplant group there are other factors that stratify cases to higher risk such as allograft dysfunction, re-transplantation, thrombocytopenia, CMV infection, technical and management issues such as anastomosis, organ rejection, and use of augmented immunosuppression. In solid organ transplantation the evolving trends include a decrease in overall incidence of candidiasis, an increase in appearance of non-albicans Candida species, and increase in mould infections such as aspergillosis with its high mortality despite treatment.

Recommendations

- Establish networks to evaluate new vaccines, new devices, new health care worker policies and other methods for the control and prevention of a range of fungal diseases.
- Validate and refine case definitions for the mycoses.
- Develop better fungal diagnostics in these high-risk patients (bone marrow transplantation and chemotherapeutic neutropenia, HIV, solid organ transplantation).
- Develop preventive and empiric strategies that may include antifungal agents for high-risk patients.
- Recognize high-risk patients as a dynamic group. Therapies such as HAART and mini-BMT may change the risks.
- Assess risk for fungal infections for both inpatients and outpatients.
- Continue surveillance and risk assessments, both on a national basis and within individual medical centers both in the community and hospital.
- Focus on preventable risk factors.
- Develop better prevention methods for fungal diseases, including vaccines, potent (fungicidal) new antifungal drugs for prophylaxis and treatment, and environmental control strategies.

COST ESTIMATES

Current Status

Nosocomial bloodstream infections caused by *Candida* species occur at a rate of 5 to 10 per 10,000 admissions to acute care hospitals, constitute 5 to 10% of all nosocomial bloodstream infections, and affect 15,000 to 30,000 patients each year in the United States. Crude or overall - mortality is at least 40% (6,000- 12,000 deaths), and the attributable or direct mortality is at least 25% (3,750- 7,500 deaths). The latter numbers reflect the direct impact of the bloodstream infections after accounting for mortality due to the underlying diseases. The same numbers also reflect the maximum opportunity for an ideal antifungal agent because even an ideal antimicrobial could impact only attributable mortality and not affect the mortality related to the underlying disease.

Historical cohort studies with tight matches of cases and controls have shown that excess hospital stay due to *Candida* bloodstream infections averages 12 days overall and twice that when only survivors are considered. The economic consequences of the attributable length of stay can be appreciated by multiplying those excess days by the expected cost per day of hospitalization. Furthermore, some of the extra stay occurs in critical care units where daily costs are two to five times those of general wards. Thus, in the managed care era, those institutions able to reduce the attack rate of nosocomial *Candida* bloodstream infections will have effectively improved their bottom line finances.

Risk factors for *Candida* bloodstream infections include colonization with the organism, central venous catheters, renal dysfunction or dialysis, and the number of antimicrobial classes to which the patient has been exposed. It remains to be shown whether the reduction of risk factors will lead to reduced infection rates. However, all are temporally related to candidemia, all are biologically plausible risk factors, and all have consistently been shown to be present among such infected patients.

Patients at risk for candidemia are found in neonatal intensive care units (low birth-weight premature babies), surgical ICUs (colon and pancreatic surgery), medical ICU's (central lines, total parentera1 nutrition and gastrointestinal disease) and bone marrow transplant units. Of interest is the fact that outbreaks with the same clones have been reported in all such units, sometimes related to contaminated fluids. More recently, it has been shown that hand carriage of *Candida* species among health care workers is much higher than previously thought, i.e., 25% or more. Further, the isolates from some infected patients match those carried by hospital staff. In future studies, hand carriage of *Candida* by healthcare personnel may be confirmed as a risk factor for infection.

In recent studies, the proportion of nosocomial bloodstream infections caused by *C. albicans* relative to non-*albicans* species has remained constant at about 50%. However, the proportions have changed among the non-*albicans* species. In the SCOPE National Surveillance System for nosocomial bloodstream infections, 50% of *Candida* isolates are non-*albicans* species. In some studies, these infections were temporally related to the use of antifungal agents, both amphotericin-B and fluconazole. Some less common species have shown resistance to the triazoles, such as fluconazole and itraconazole. Fortunately, new oral antifungal agents with potent antifungal activity are being investigated.

The cost of fungal infections, specifically nosocomial candidemia, was estimated in a study using treatment incidence, i.e., the extent or frequency that patients seek care for the disease. This study examined the marginal increase in direct medical costs associated with treating candidemia diagnosed during a hospital stay in the U.S. The study design was a cost-of-illness analysis estimating the average cost of candidemia for a single episode of care. Data were obtained from the 1990 National Discharge Data Survey, 1990 National Health Interview Survey, and the 1993 Healthcare Cost and Utilization Project. The estimated cost of an episode of care for candidemia was $34,123 (1997 US dollars) per Medicare patient. The major cost associated with candidemia is an increased hospital stay. Strengths of the study include the use of actual hospital data and use of controls to determine marginal, incremental differences. Limitations of the study were that it was a hospital-based study conducted retrospectively and only direct medical costs were included resulting in underestimates of national costs. Also, cost of recurrence was not included in this study. Cost studies for the mycoses provide quantitative evidence as to the importance of medical mycoses, assist in planning data collection needs in intervention trials, generalize clinical trial data to "real world" practices, evaluate rare or difficult to measure effects or resource utilization, examine long-term effects that are not feasible to evaluate in intervention trials, assess the impact of changes in key cost or outcome variables on overall results, evaluate costs and cost-effectiveness versus comparators not included in intervention trials, and provide justification for expensive tests, procedures, and therapy to healthcare decision makers.

Recommendations

- Develop more effective diagnostic tests for the invasive mycoses not only to improve treatment outcome but to ascertain the real incidence and attributable costs.

- Develop cost estimates from NCHS surveys and other relevant databases

- Continue to assess health care costs attributable to fungal infections in complex hosts, procedures (e.g., bone marrow and transplantation and other surgical interventions), and anti-immunosuppressive procedures.

- Work with mycologists and public health officials in other countries to determine the global incidence and attributable costs of fungal infections given that *Aspergillus* and *Candida* are likely to be globally emerging pathogens linked to medical advances.

GENETIC AND BIOLOGIC VARIATION IN THE FUNGUS AND HOST

Current Status

The tools of molecular evolutionary biology and genomics are making it possible to use genetic variation in pathogens and hosts to prevent and treat mycoses. The first important area is the detection of pathogens responsible for opportunistic disease. Variation in fungal pathogen genotype is the basis for developing methods to identify these pathogens using PCR. Recent work at Roche Molecular Systems has used the most variable region of the SSU 18S rDNA to identify *Aspergillus* and *Candida*. With *Candida*, as many as 30 probes are used in a line-blot format permitting identification of 11 Candida species from blood, including *Candida albicans* and non-*Candida abicans* species. With *Aspergillus*, 14 species can be detected from broncheal alveolar lavage, sputum, lung biopsy, or blood. However, the extreme sensitivity of these PCR tests and the presence of both *Candida* species and *Aspergillus* species in the environment or on lab personnel, makes manufacture of contaminating DNA-free reagents difficult. This challenge raises the potential for false positive diagnoses.

The second important area is the use of molecular population genetics to recognize cryptic species and to uncover reproductive mode. Recent molecular population genetic studies of human pathogenic fungi such as *Candida, Coccidioides* and *Histoplasma* have recognized new species and found that fungi thought to be asexual are recombining in nature as well as reproducing clonally. Knowledge of genetic differentiation and isolation is important for disease prevention via vaccination, because vaccines must be effective against proteins that are polymorphic among species. Finding that an asexual fungus is recombining affects identification strategies because of the mixing of markers due to recombination. For *Candida albicans*, population genetic evidence of recombination in nature may have been explained by two recent reports of mating and recombination in the laboratory. With *Cryptococcus neoformans*, genetic isolation among serotypes A, D and B+C has been shown to be as great as between species of other fungi, and there is evidence of hybridization between individuals of serotypes A and D.

The third important area is the relationship between environmental and clinical isolates of human pathogenic fungi. RAPD-PCR studies with primers for different genes were performed on *Histoplasma capsulatum* isolates recovered from adult bats and bat guano in Mexico and from patients from states in the central zone of the Mexican Republic and Guatamala. Clinical isolates from all areas showed less variation than environmental isolates, but were identical to some environmental isolates in the same localities. These findings suggest that clinical cases arise from isolates that are capable of infecting bats, and that bat-mediated transportation of *H. capsulatum* is keeping populations in Mexico and Central America from diverging.

The fourth important area is the use of genetic variation in host genotype to prevent disease by identifying susceptible host populations. In infectious disease pathogenesis, molecules that control the immune response can determine the outcome. If the genes encoding these molecules or their expression are variable, this variation can determine the course of disease. Studies to investigate host genetic risk factors for fungal disease are sparse. Recently, genetic epidemiological techniques were used to examine how host genetic variation influences the severity of coccidioidomycosis. In a case-control study, Caucasian, Hispanic, and African-American patients with mild or disseminated disease were compared with population controls. ABO blood group was associated with disease among Hispanics. Some HLA class I and II alleles and haplotypes were associated with risk of disease and were shared among the ethnic groups. However, most associations differed by ethnic group. Overall, the mild and severe cases differed from controls but not from each other. The role of other factors and genes involved in susceptibility to severe coccidioidomycosis will be uncovered as the molecular mechanisms of the immune response in fungal pathogenesis becomes further known.

The fifth important area is the monitoring of host response to disease and treatment may become possible using mRNA profiling. Variation in host genotype is supporting genome wide profiling which is leading to identifying host groups at risk for disease, monitoring markers that are surrogates for host response to disease and treatment, and using peripheral blood cells to interrogate the condition of deeper host tissues and organs. mRNA expression levels can be monitored by arrays or kinetic RT-PCR. Arrays can score more mRNAs but kinetic RT-PCR is more sensitive and has a greater dynamic range. Kinetic RT-PCR is being used to profile chemokines, cytokines and receptors found in PBMCs and is showing that mRNA levels vary among individuals and are stable over time per individual but are variable when the host disease state changes. Technically, monitoring host genetic variation is becoming increasingly useful but social hurdles involving regulation and acceptance can be expected and will be difficult to overcome.

Recommendations

- Develop a standard, rapid, and reliable way of identifying fungal pathogens.
- Move toward looking at fungal genotypes for clinical and biological relevance.
- Generate more data that can be compared across fungal species (i.e., sequence data, not RAPDs).
- Consider a pilot program focused on a set of fungi causing either endemic or opportunistic mycoses. Such a pilot could examine key genetic factors from the pathogen and the host from which it was derived.
- Consider creating "FungusNet," a worldwide web accessible database modeled after PulseNet (CDC) which would contain all the information associated with an isolate and its genotype.
- Increase efforts to complete fungal genome sequences for the systemic pathogens, *Coccidioides immitis* and *Histoplasma capsulatum*, for comparison with ongoing projects for the opportunistic pathogens, *Candida albicans, Cryptococcus neoformans, Pneumocystis carinii* and *Aspergillus fumigatus*.

LABORATORY METHODS FOR STRAIN IDENTIFICATION

Current Status

It is now standard practice for epidemiological studies of infectious diseases to require that pathogens be characterized to the subspecies level whenever possible to better define the infectious processes and modes of transmission. Although many different typing methods have been used in epidemiological studies of fungal pathogens, the DNA-based molecular typing (DNA fingerprinting) methods have been most useful for this purpose.

Molecular epidemiologic typing systems are used to assist the epidemiologist and microbiologist in answering the question of whether two or more isolates of a given species of microorganism are "indistinguishable" or "different". The question may be raised in epidemiological investigations of clusters of infections, in larger population survey studies, in the management of individual patients, or in studies of pathogenesis. A broad range of typing methods has been used to generate molecular fingerprints of different fungi and the method used in any given study may vary with the organism and specific goals of the investigation. The methods include, but are not limited to: restriction fragment length polymorphism (RFLP) analysis using specific probes and Southern hybridization analysis, restriction endonuclease analysis of genomic DNA (ethidium bromide staining), pulsed-field gel electrophoresis (PFGE) and electrophoretic karyotyping, restriction endonuclease analysis with rare cutters and PFGE (macro-restriction digest), and numerous variations of PCR-based fingerprinting.

In a typical epidemiological investigation, isolates from two or more patients are examined in order to determine whether the infections are due to strains which are indistinguishable or different. In most instances, if the isolates are classified as different by at least one molecular typing method, they may be assumed to represent different strains and to reflect independent infections. Minor changes in the mobility of one or two bands may reflect microevolution within the strain and may have pathogenic or drug resistance implications. If the isolates are indistinguishable, it is likely that cross-infection has occurred or that the patients were infected by exposure to a common source. DNA fingerprinting methods may also be used to address clinical problems such as distinguishing reinfection from relapse and to examine the course of development of antifungal resistance among fungal isolates obtained during the course of therapy. Sequential isolates of a single species of fungus obtained from an individual patient may be tested to detect strain relatedness. Recovery of the same strain on multiple occasions suggests the possibility of a relapsing infection, possibly due to a residual focus of infection such as a catheter or an undrained abscess, whereas repeated infections with different strains of an organism may suggest that the patient is predisposed to infection as a result of specific exposures or host defects. Similarly, determination of DNA fingerprints of sequential isolates from patients undergoing antifungal therapy has been useful in demonstrating the potential for the development of antifungal resistance in previously susceptible strains and for detecting the substitution of a more resistant strain for a more susceptible strain in circumstances where selection by antimicrobial pressure is an issue. Finally, the linking of environmental strains of organisms such as *Pneumocystis carinii* and *Aspergillus fumigatus* with those causing pulmonary infection in patients has been important in our understanding of disease processes due to these organisms.

DNA fingerprinting of fungal pathogens may be accomplished using a variety of different methods as noted above. In most instances DNA fingerprinting methods involve comparisons of banding patterns which are assumed to reflect genetic relatedness and are generated by some form of electrophoresis. In order to be useful as an epidemiological typing method, a DNA fingerprinting method must be reproducible, must distinguish between genetically unrelated strains, be capable of identifying the same strain in different samples, and reflect genetic relatedness or unrelatedness (genetic distance) among strains or species. When studying large collections of organisms it is also important that the banding patterns produced by the typing method are amenable to computer analysis. Although the ability of most of the DNA fingerprinting methods used at present to measure genetic distance has not been established, qualitative analysis of the DNA banding patterns generated by these methods has been useful in studies of several fungal pathogens.

Molecular epidemiological typing methods have clearly played an important role in our improved understanding of the epidemiology of several fungal infections. Almost irrespective of the DNA-based typing method employed, the general findings are the same: 1) fungi, especially *Candida* spp., may be transmitted in hospital just like other nosocomial pathogens; and 2) the major reservoir for most systemic yeast (e.g. *Candida* spp.) infections is the patient's own flora and the environment is the main source of strains of *P. carinii* and *Aspergillus* spp. strains causing human infection. It is now time to refine these methods and to conduct studies that may give us an even more precise and detailed understanding of the differences (if any) between commensal strains colonizing healthy non-hospitalized individuals and those strains isolated from the bloodstream and tissues of infected, hospitalized patients. These studies will require careful attention to both the collection of isolates from appropriate patient populations (controls as well as patients) and to the methods used for DNA fingerprinting and the analysis of the resulting DNA profiles. It is important that the ability of the fingerprinting methods used to distinguish genetically related and unrelated strains be validated, that the methods provide quantitative information that reflects genetic distance, and that the patterns are amenable to computer-based analysis. The information gained from such studies may provide new insights into the acquisition of potentially more pathogenic strains of fungi and the means to prevent such acquisition.

Recommendations

- Standardize DNA fingerprinting methods and interpretation as they pertain to pathogenic fungi.
- Validate DNA fingerprinting methods used in molecular epidemiologic studies against one or more methods known to be acceptable measures of genetic distance.
- Include control strains (from uninfected patients) with each molecular epidemiology study of pathogenic fungi.

TOPIC SUMMARY AND SPEAKERS

Overview of the Mycology Workshop Series

Dennis M. Dixon, Ph.D. National Institutes of Health

Surveillance

Chairperson: David Warnock, Ph.D. Centers for Disease Control and Prevention
Robert W. Pinner, M.D. Centers for Disease Control and Prevention
Michael A. Pfaller, M.D. University of Iowa College of Medicine
Rana A. Hajjeh, M.D. Centers for Disease Control and Prevention
Roderick J. Hay, DM FRCP FRCPath St. John's Institute of Dermatology

Risk Factor Studies

Chairperson: John Perfect, M.D. Duke University Medical Center
Thomas J. Walsh, M.D. National Cancer Institute
William G. Powderly, M.D. Washington University School of Medicine
Nina Singh, M.D. VA Medical Center, Pittsburgh

Cost Estimates

Chairperson: George Kobayashi, Ph.D. Washington University School of Medicine
Richard P. Wenzel, M.D. Medical College of Virginia
Anne M. Rentz, M.S.P.H., R.D.H. Medtap International

Genetic and Biologic Variation in the Fungus and Host

Chairperson: John Taylor, Ph.D., University of California at Berkeley
Thomas J. White, Ph.D., Applied Biosystems, Perkin-Elmer, Foster City, CA
Rytas Vilgalys, Ph.D., Duke University
Maria Lucia Taylor, Ph.D., UNAM, Mexico City
Leslie Louie, Ph.D., M.P.H., Children's Hospital Oakland Research Institute, Oakland, CA

Laboratory Methods for Strain Identification

Chairperson: Michael A. Pfaller, M.D. University of Iowa College of Medicine
David R. Soll, Ph.D. University of Iowa
Melanie T. Cushion, Ph.D. University of Cincinnati College of Medicine
Alex van Belkum, Ph.D. University Hospital Dijkzigt, The Netherlands

Facilitators

Beth DiDomenico, Ph.D. Schering-Plough Research Institute
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